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(71)(72) Applicants and Inventors: BALAZOVSKIY, Mark Borisovich [RU/RU]; ul. Nalichnaya, 3/21-90, St.Petersburg, 199106 (RU). KOZHEMYAKIN, Leonid Andreevich [RU/RU]; Pulkovskoe shosse, 13/2-5, St.Petersburg, 196240 (RU).

(74) Agent: SPESIVTSEVA, Irina Jurievna; Neopharm Ltd., pr. Energetikov, 37, St.Petersburg, 195248 (RU).

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(54) Title: CYTOKINE AND HEMOPOIETIC FACTOR ENDOGENOUS PRODUCTION ENHANCER AND METHODS OF USE THEREOF

(57) Abstract

The invention concerns medicine, in particular pharmacology and therapy, and can be used in treatment of oncological, immunological, infectious, hematological and other diseases, where it is expedient the stimulation of endogenic cytokines production, reproduction of biological effects of cytokines and hemopoietic factors. According to the invention, as stimulator of endogenic cytokine production, effects of cytokines and hemopoietic factors, it is offered to use oxidized glutathione representing dimer with structure of reduced glutathione with γ-glutamyl-cysteinilglycine structure, in which two molecules of peptide are connected with each other by covalent bisulfidic bound between cysteine residues, and also various pharmaceutic compositions with its participation, mainly, consisting from the medicinal form of oxidized glutathione and pharmaceutically acceptable component, capable to prolong staying of oxidized glutathione at its introduction in biological media, in oxidized form.

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CYTOKINE AND HEMOPOIETIC FACTOR ENDOGENOUS PRODUCTION ENHANCER AND METHODS OF USE THEREOF

Field of the Invention

The present invention relates to medicine and more particularly to pharmacology and therapy, and is intended to be used for preventing and treating various diseases by way of increasing endogenous production of cytokines and hemopoietic factors

Background of the Invention

It has been known that a number of endogenously produced mammalian humoral factors — cytokines and hemopoietic factors — possess important biological activities that are considerably helpful in treating various human diseases[1,2]. Many of these factors are being tested in man, those with proven efficacy being commercially available as medicinal agents.

The following cytokines and hemopoietic factors are being most extensively researched in oncology: interleukin 2 (IL-2)[3,4], tumor necrosis factor alpha (TNF-α)[5], erythropoietin, macrophage-granulocyte and granulocyte colony-stimulating factors (GM-CSF and G-CSF, respectively[6,7]). No less actively is being studied the use of cytokines and hemopoietic factors for the treatment of infectious disease: interferons (IFN-γ and IFN-β)[8,9,10], colony-stimulating factors[11,12], and the like[13]. Colony-stimulating factors and erythropoietin are broadly used in hematology[14,15]

However, the medicinal use of these exogenously administered agents has its limitations associated with the lack of acceptable drug formulations or their exorbitant cost, a short half-life of these substances in biological media, difficulties in dose finding as well as numerous toxic and allergic effects[16,17], since even the recombinant products are more or less immunogenic to the human organism because of the processing

In this regard, in the view of achieving a more invariable and significant therapeutic effect free of adverse reactions, it is preferable to induce the endogenous production of

the autologous cytokines and hemopoietic factors immediately within the organism of a subject. The remedial effect due to such intrinsic stimulation is free of all the disadvantages associated with exogenously introduced cytokines and hemopoietic factors.

A number of compounds are currently being evaluated that stimulate endogenous production of cytokines and hemopoietic factors in both experimental and clinical settings. There are universally known cases, including successful ones, of using microbial products for cancer therapy which in recent decades has been shown to be mediated via stimulation of the tumor necrosis factor endogenous production[18]. The products capable of evoking concomitant production of various cytokines and hemopoietic factors have presently come to be known as multi-cytokine inducers. Among these are a killed streptococcal preparation, *Nocardia opaca*, and other bacterial products[19, 20, 21]. However, virtually all the substances possessing such capability are either killed microorganisms or microbial products or compounds having irregular composition, which results in their limited medicinal utility or even renders their therapeutic use impracticable. Thus, the problem of finding a medically and pharmaceutically acceptable inducer of the cytokine and hemopoietic factor endogenous production has not heretofore been resolved.

Having performed studies in search for a medically and pharmaceutically acceptable inducer of the cytokine and hemopoietic factor endogenous production, the applicant discovered a new property of a previously known substance — oxidized glutathione (oxidized glutathione, glutathione disulfide, GSSG, hereinafter referred to as GSSG). Being administered parenterally or acting on isolated cells, the substance is capable of inducing production of several cytokines and hemopoietic factors in mammals (laboratory animals and humans) in both health and disease.

GSSG is known as a dimer of tripeptide glutathione (γ-glutamyl-cysteinyl-glycine) [illegible] covalent disulfide bond between the cysteine residues. Therefore, both the tripeptide glutathione (glutathione, reduced glutathione, GSH, hereinafter referred to as GSH) and its dimer GSSG are natural metabolites present in animal and human tissues and

biological fluids. At the same time, the natural blood level of GSSG is not sufficient for inducing the cytokine endogenous production in both normal and pathological conditions.

GSH is known to be one of the most important intermediates in the amino acid metabolism and a factor maintaining the intracellular homeostasis[22, 23]. The reducing properties of GSH and its function as a donor of reduction equivalents, which is due to the sulfhydryl moiety of the cysteine residue, are of key importance. This characteristic of GSH is responsible for the substance playing a crucial part in one of the most important intracellular antioxidant systems, consisting of GSH as such and two enzymes of its reversible conversion into GSSG: glutathione peroxidase and glutathione reductase[24, 25]. The permanent functioning of said system is essential for inactivating or reducing endogenously generated oxidants as well as active metabolites of foreign substances[26, 27].

GSH is also known to participate in detoxification reactions involving a group of enzymes collectively known as glutathione S-transferase[28]. These enzymes are capable of conjugating the GSH molecule with various xenobiotics by forming a bond between the latter and glutathione via the thiol moiety of the cysteine residue of the tripeptide. The subsequent degradation of the conjugate is catalyzed by the γ-glutamyl cycle enzymes, and may vary considerably depending upon the nature of the xenobiotic.

Under natural conditions, GSSG does not accumulate in amounts sufficient for inducing cytokine and hemopoietic factor production, due to a constant reduction of GSSG to GSH. The GSSG reduction to GSH also actively progresses in the intestines and liver upon GSSG oral administration, and as any product made of amino acids, the substance is proteolytically degradable in the gastrointestinal tract.

GSSG is known to be used as a component of a nutritional supplement utilized as an adjunct diet in treating patients[29]. However, being a peptide substance, most of the orally administered GSSG is digested in the gastrointestinal [illegible] being reduced in the intestinal and hepatic cells to GSH and not entering the circulation. Therefore, the delivery of GSSG into the organism through the

gastrointestinal tract eliminates the possibility of the realization of its activity as an stimulator of endogenous production of cytokines and hemopoietic factors

An elevation of the GSH endogenous levels for medicinal utility is known to be suggested for boosting immunity[30] and treating toxemias, poisonings, diabetes mellitus, cardiovascular, infectious and other disorders[31, 32, 33].

Exogenous GSH or its direct (γ-glutamyl-cysteine, *n*-acetyl-cysteine, and *n*-acetyl-cysteinyl-glycine) or indirect (2-oxothiazolidine-4-carboxylate) biochemical precursors, or their salts and esters, are reportedly used as medicinal agents and dietary supplements in treating various diseases The use of such substances and their compositions is suggested to increase the endogenous GSH level for the treatment of various diseases and toxemias[34, 35, 36, 37, 38].

GSH is also claimed to be useful as a chemoprotective agent that prevents neurotoxicity in cancer chemotherapy[39] as well as in combination with antineoplastics in order to augment their effect[40].

No reference, however, is currently available to GSSG as a medicine in its own right (sole substance) used to induce the endogenous production of cytokines and hemopoietic factors. The substance is known neither to have medicinal effects in human and animal diseases nor to be applied as a pharmaceutical agent for treating illnesses.

Summary of the Invention

It is an object of the present invention to provide an active substance capable of inducing the endogenous cytokine and hemopoietic factor production in subject in need thereof

"Subject in need thereof" is intended to mean a mammal, *e.g.* man, domestic animals [illegible] the endogenous cytokine and hemopoietic factor production would be considered beneficial by those skilled in the art

In accordance with the present invention, it is GSSG that upon parenteral administration induces the endogenous cytokine and hematopoietic factor production in subject in need thereof, in both health and disease

In accordance with the present invention, an inducer of the endogenous cytokine and hemopoietic factor production is oxidized glutathione (GSSG) which is a dimer of reduced glutathione having the structure γ-glutamyl-cysteinyl-glycine, where the two molecules of the tripeptide are linked via a covalent disulfide bond between the cysteine residues.

The applicant has for the first time shown that an immediate action of exogenous GSSG on mammalian (human and laboratory animal) cells capable of producing cytokines or hemopoietic factors, exerts stimulation on the synthesis of these molecules and their release into the blood and microenvironment, which results in their increased level in the blood serum (*in vivo* conditions) or culture media (*in vivo* and *ex vivo* conditions). The method suggested can bring about the effect of stimulating production of cytokines and hemopoietic factors, and this effect is elicited by the administration of GSSG into the organism or entering into the cultural media, as well as by the administration of GSSG in the composition of pharmacologically active formulations mediating the prolongation of glutathion's retaining the oxidized form. The studies performed by the applicant have revealed GSSG and its formulations to possess a therapeutic effect in various experimental and clinical pathological conditions.

The applicant suggests that the revealed GSSG-induced stimulation of the endogenous cytokine and hemopoietic factor production underlies antitumor, antiinfective, hemopoietic, immunomodulatory and other pharmacological effects resulting, in turn, to a greater or lesser extent therapeutic or preventive effect in various diseases.

In accordance with the present invention, the medicinal agent suggested for treating neoplastic, infectious, hematologic, and other diseases, in which stimulation of the endogenous cytokine and hemopoietic [illegible] effective amount of GSSG as its active principle. It is also advantageous to prepare

the drug form of the medicinal agent as an injectable solution containing 0.01 to 2.0% GSSG.

In accordance with the present invention, it is expedient to use such GSSG drug forms and/or pharmaceutical compositions that prolong oxidized glutathione half-life in tissues and biological fluids or augment the revealed biological and therapeutic properties of GSSG.

In accordance with the present invention, with the purpose of augmenting and prolonging the therapeutic effect of GSSG, its drug form (injectable solution) is suggested to contain a pharmaceutically acceptable component capable of extending the oxidized glutathione half-life.

As a pharmacutical acceptable component to prolong glutathione permanence in oxidized form, 0.003% hydrogen peroxide can be offered for application. This is because in the presence of hydrogen peroxide, a donor of reactive oxygen intermediates (that is an oxidant), GSSG is reduced by glutathione reductase to GSH at a lesser speed, thereby conditioning a slower reduction of GSSG introduced exogenously into biological media.

Usage of an acceptable concentration of hydrogene peroxide (H_2O_2) in formulation of the drug form for parenteral administration, as well as usage of any other prooxidant compounds (donors of active oxygen form), makes it possible to realize only one of possible methods of the prolongation of oxidized glutathione half-life in the biological fluids and tissues and, thereby, to enhance and prolong the pharmaceutical effect of GSSG.

We have also found some other pharmaceutically acceptable components capable of mediating the slowdown of the reduction of exogenous GSSG into GSH in biological media. Such, in particular, are the factors capable of setting up competitive relations

[illegible]

agents reversibly inhibiting the processes of reduction of the oxidized form of NADP ·

into NADP•H, for example, cystamine (2,2'-Dithio-bis[ethylamine]) and other inhibitors of glucose-6-phosphate-dehydrogenase.

Since reduced NADP•H is the key cofactor of glutathionereductase system catalyzing the reduction of GSSG into GSH, any pharmaceutically acceptable compounds or biophysical influence retarding the reduction of GSSG or blocking biological oxidation of NADP•H by glutathionereductase will facilitate preservation of GSSG from reduction in biological media and, therefore, will enhance and prolong its curative effect.

Due to conducted research we were the first to discover that GSSG pharmaceutical and medicinal effect will reinforce, when GSSG used in combination with agents capable of competition with NADP•H, as well as with compounds reversibly inhibiting the enzymatic reaction, catalyzed by glucose-6-phosphate-dehydrogenase which mediates the reduction of the oxidized form of NADP+.

Thus, besides hydrogen peroxide, one of other pharmacologically accepted components capable to prolong the oxidized glutathione half-life can be inosine (hypoxanthie-9-D-ribofuranoside) used as 0.1% solution.

The investigations carried out showed inosine to facilitate biological and therapeutical effects of GSSG. It was demonstrated that this property of inosine is based on its ability to compete with NADP•H, and thereby, to retard GSSG reduction into GSH. Moreover, we have also found that other hypoxanthine derivatives (including inosine nucleoside ones) possess this property as well.

Also, beside hydrogen peroxide and inosine, cystamine (2,2'-Dithio-bis[ethylamine]) is another pharmaceutically acceptable agent conditioning a slower reduction of GSSG, if used as 0.1% solution.

[illegible]

[illegible] enzyme of the pentose phosphate pathway, glucose-6-phosphate-dehydrogenase, mediating reduction of NADP+ into NADP•H.

Thus, the present invention suggests the method to enhance the ability of GSSG to stimulate endogenous production of cytokines and hemopoietic factor which presupposes the usage a pharmaceutical composition including GSSG and an additional component able to prolong the oxidized glutathione half-life. This can be achieved by the administration of pharmaceutically acceptable compositions including <u>drug forms</u> of GSSG and <u>drug forms</u> of other products, able to prolong the oxidized glutathione half-life, such as: 0.003% hydrogen peroxide or other compounds with oxidant activity; 0.1% inosine (hypoxanthie-9-D-ribofuranoside) or its derivatives including inosine nucleosides; and also 0.1% cystamine (2,2'-Dithio-bis[ethylamine]) or other compounds, capable to produce reversible inhibition of glucose-6-phosphate-dehydrogenase, the key enzyme of the pentose phosphate pathway.

It is found that the parenteral (intravenous, intraperitoneal, intramuscular, *etc.*) administration of GSSG in 0.003% solution of hydrogen peroxide, or GSSG in 0.1% inosine solution, or GSSG in 0.1% cystamine solution stimulates endogenous production of TNF-α, IFN-α and IFN-γ, IL-1, IL-2, IL-6, IL-10, and GM-CSF in organism of experimental animals in a larger degree than with the application of GSSG alone.

The studies carried out prove the ability of the above mentioned compounds to enhance the biological and therapeutical effects of GSSG, which makes evident the expediency of their use in combination with GSSG to treat neoplastic, infectious, hematological and other diseases in which stimulation of the endogenous cytokine and hemopoietic factor production is considered beneficial by those skilled in the art.

Thus, in accordance with the present invention, for the purpose of enhancing and prolonging the GSSG therapeutical effect, it is expedient that a final drug formulation (1-5 ml of solution for injections) should contain additional pharmaceutically acceptable components able to prolong the oxidized glutathione half-life in the [illegible]

a) 0.003% hydrogen peroxide or any other pharmaceutically acceptable pro-

oxidant compounds with activity of the donors of reactive oxygen intermediates,

b) 0.1% inosine (hypoxanthie-9-D-ribofuranoside) or any other pharmaceutically acceptable competitors of NADP•H-dependent reduction of GSSG into GSH catalyzed by glutathione reductase

c) 0.1% cystamine (2,2'-Dithio-bis[ethylamine]) or or any other pharmaceutically acceptable compounds able to produce reversible inhibition of reduction of NADP+ into NADP•H catalyzed by glucose-6-phosphate-dehydrogenase or by other NADP•H-dependent enzymes.

At the same time, the data were obtained to testify the direct antitumor effect of GSSG, or GSSG administered together with the pharmaceutically acceptable compounds prolonging oxidized glutathione half-life in biological media. Moreover, the GSSG effect was proved to be different for normal and tumor cells. Our in vitro research with the use of normal and tumor cells revealed that the GSSG alone, or its pharmaceutically acceptable compositions containing compounds prolonging oxidized glutathione half-life in biological media, initiated tumor cell death in apoptosis like manner. In case of normal cells, they did not undergo destruction.

It is an object of the present invention to provide a method for treating neoplastic, infectious, hematologic and other diseases in which stimulation of the endogenous cytokine and hemopoietic factor production is advantageous. The method comprises parenteral administration of GSSG as <u>the medicinal agent</u> in the injectable <u>drug form</u> at 0.01 to 0.5 mg GSSG per kg body weight, one or more times a day, by one or more day pulses or continuously until the therapeutic effect has been achieved.

It is essential that either GSSG as <u>medicinal agent</u> or its <u>drug forms</u> and/or pharmaceutical compositions be administered strictly parenterally so that to prevent or [illegible] tract upon oral administration.

Provided GSSG molecule is protected from proteolysis and/or reduction to GSH, it

would be advantageous to administer the agent orally and/or intralesionally (*in situ*) (wound, tumor, *etc.*). In addition, the treatment method of the invention, with the purpose of augmenting or prolonging the effect of the medicinal agent, may include a concomitant physical intervention, *e.g.*, UV- or X-ray, if such intervention is capable of extending the oxidized GSSG half-life in tissues and biological fluids.

The examples given below confirm that the parenteral (intraperitoneal, intravenous, intramuscular, subcutaneous, *etc.*) use of GSSG results in inducing the endogenous production of TNF-α, IFN-α and INF-γ, IL-1, IL-2, IL-6, IL-10, erythropoietin, and GM-CSF in mammals, which elicits a significant therapeutic effect in animals and humans suffering from neoplastic or infectious disease, hemopoiesis and immunity suppression of different origin, and other diseases in which stimulation of the endogenous cytokine and hemopoietic factor production would be considered beneficial by those skilled in the art.

From the experimental findings (see Examples) it follows that the previously unknown GSSG capability of inducing the endogenous cytokine and hemopoietic factor production and exerting beneficial effects in various diseases, is not associated with an increase in GSH levels, because GSH testing in a wide range of doses and concentrations has revealed neither stimulation of the endogenous cytokine and hemopoietic factor production nor the therapeutic effect observed with the use of GSSG.

As used herein, the term "therapeutic effect" means any improvement in the condition of a patient or animal treated according to the subject method, or any alleviation of the severity of signs and symptoms of a disease and its sequelae, including those caused by other treatment methods (*e.g.*, chemo- and X-ray therapy), which can be detected by means of physical examination, laboratory or instrumental methods and considered statistically and/or clinically significant by those skilled in the art.

As used herein, the term "prophylactic effect" means prevention of any worsening in the condition of a subject treated according to the method of the invention, as well as prevention of any exacerbation of the severity of signs and symptoms of a disease or its

sequelae, including those caused by other treatment methods (*e.g.*, chemo- and X-ray therapy), which can be detected by means of physical examination, laboratory or instrumental methods and considered statistically and/or clinically significant by those skilled in the art.

As used herein, the terms "neoplastic and infectious disease", "hemopoiesis and immunity depression of various origin", and "other diseases" mean any neoplastic and infectious disease, any condition caused or accompanied by the erythroid or myeloid suppression, or a reduction in quantitative or functional immunity parameters, as well as any other disease or pathological condition in which stimulation of the endogenous cytokine and hemopoietic factor (TNF-α, IFN-α and INF-γ, IL-1, IL-2, IL-6, IL-10, erythropoietin, and GM-CSF) production would be considered advantageous by those skilled in the art.

Detailed Description of the Preferred Embodiment

The examples given below demonstrate feasibility of the invention.

The active principle, the GSSG peptide capable of inducing the endogenous cytokine and hemopoietic factor production, may be obtained by conventional peptide synthesis techniques[41].

Thereby obtained peptide (GSSG) is subsequently used in animals and humans (*in vivo*) as the GSSG base, or as a pharmaceutically acceptable GSSG salt, in an injectable drug form prepared by dissolving the bulk substance in injectable water, or in any pharmaceutically acceptable solvent, with the resultant concentration of the active compound being 0.01-2.0%.

For an *in vitro* use in experimental settings, GSSG may be dissolved in biologically acceptable solvents — culture media, isotonic saline solutions, and the like.

[illegible] pyrogen-free conditions while exerting every effort to prevent chemical or bacterial contamination.

The GSSG injectable drug form has been tested in both animal studies and pilot human trials

The use of the maximum achievable concentration of the GSSG sodium salt solution (10.0%, 100 mg/mL) in injectable water (or in normal saline, or in 0.003% hydrogen peroxide, or in 0.1% inosine, or in 0.1% cystamine), and the maximum tolerable volumes administered to mice intraperitoneally (IP, 2.0 mL), intravenously (IV, 0.5 mL), and intramuscularly (IM, 0.05 mL), have made it feasible to reach GSSG dosage levels 5000 mg/kg (IP), 1350 mg/kg (IV), and 135 mg/kg (IM), *i.e.* 1000, 270, and 27 times, respectively, the maximum recommended human dose of 0.5 mg/kg. In none of the cases either animals' deaths or any toxic signs were observed, showing GSSG in injectable drug form to be essentially nontoxic.

The results of nonclinical evaluation of biological, pharmacological, and therapeutical properties of GSSG, as well as its drug forms with or without 0.003% hydrogen peroxide, or 0.1% inosine, or 0.1% cystamine, are presented in Examples ## 1–5.

Example #1

Effect of GSSG and its drug forms on cytokine production by human peripheral blood mononuclear leukocytes in vitro

Oxidized glutathione (GSSG), as well as its drug forms containing 0.003% hydrogen peroxide, or 0.1% inosine, or 0.1% cystamine, were evaluated for their effect on cytokine production by human peripheral blood mononuclear leukocytes *in vitro*

The leukocytic cytokine production was triggered by adding a mitogen, concanavalin A (ConA) to the cell culture immediately after introducing the test substances. In 24 hours of the cellular exposure to ConA and the test articles, the culture supernatants were sampled and stored until cytokine determination at -70°C

[illegible] responding to the mitogen in the presence of the test articles at each concentration level, the control cell cultures, containing the test articles in identical concentrations,

were incubated for 72 hours following the initial concomitant introduction of ConA and the test substances. 16 hours prior to the incubation completion, ^{3}H-thymidine was added, and the label rate of incorporation into DNA was interpreted as the criterion of the cellular test system functional state.

Venous blood from male healthy volunteers was collected into plastic heparinized tubes (endotoxin tested) PMNL fraction was isolated by centrifugation in density gradient of Ficoll and sodium diatrizoate (Histopaque-1077; Sigma).

Cell concentration was adjusted to 2×10^6 per mL of "complete" culture medium (RPMI 1640, Sigma) containing: HEPES (20 mM); L-glutamine (2 mM); Gentamicin (50 μg/mL); fetal calf serum (10%). All the reagents used were of "cell culture tested" grade, Sigma Cell viability was estimated by the Trypan blue exclusion method and 100 μL of cell suspension (200 000 cells) was placed into each well of flat bottom 96-well sterile microtiter plates for tissue cultures. Cells from each subject were placed into no less than 39 wells.

The five following final concentrations of the test articles (GSSG, as well as its drug forms containing 0.003% H_2O_2, or 0.1% inosine, or 0.1% cystamine) were evaluated: 5000 μg/mL; 500 μg/mL; 50 μg/mL; 5 μg/mL; and 0.5 μg/mL. Each concentration was established in no less than 6 wells by adding 50 μL of "complete" medium containing the appropriate quantity of the previously dissolved test articles. Another 6 wells were used for control cultures and contained no GSSG: 50 μL of "complete" medium, or correspondingly, "complete" medium containing 0.003% H_2O_2 or 0.1% inosine, or 0.1% cystamine, were added.

Immediately after the test articles had been entered into the cultures, 50 μL of "complete" medium containing ConA (Sigma, cell culture tested) in a quantity required for a final concentration of 4.0 μg/mL, was added to all the wells excepting 3 additional ones which served for evaluation of spontaneous ^{3}H-thymidin uptake (without ConA).

After a twenty four hour incubation at 37°C [illegible] each sextuplet of identical wells were taken [illegible] the supernatant were frozen and kept at -70°C until the cytokine assay. Cultures in the other 3 wells (of each sextuplet) were incubated further under the conditions described above

Fifty six hours after the incubation had begun, 1.0 μCi of ^{3}H-thymidin was added into all the remaining cultures, the plates were incubated for another 16 hours, and then the contents of the wells were harvested and transferred onto glass-fiber filters which were consequently treated with 5% trichloroacetic acid and ethanol. The filters were dried and their radioactivity (counts per minute, cpm) was determined using liquid scintillation counter, Betaplate 1205 (LKB).

Mean radioactivity values for triplicates of identical cultures were used to calculate the index of mitogenic stimulation: the ratio of averaged cpm values of ConA stimulated cultures to averaged cpm values of nonstimulated ones (3 wells without ConA). This stimulation index for wells, where the test articles were present in various concentrations, served as a criterion of cellular culture functional status, and ability of the cells to respond to mitogenic stimulation.

Supernatants of 24-hour culture triplicates were subsequently assayed for cytokine content only if their 72-hour matched control culture triplicates developed mitogenic response to ConA with value of the stimulation index in the range from 15 to 50.

Concentrations of interleukin-1b (IL-1b), interleukin-6 (IL-6), tumor necrosis factor α (TNFα), and interferon α (IFNα) were determined by ELISA using commercial reagent kits (Medgenix, Belgium) and were expressed in pg/mL of culture supernatants.

The salient findings are given in Tables 1-4. As can be seen from Tables 1 and 2, the adding of GSSG into the culture media resulted in statistically significant and dose-dependent stimulation of the cytokine production by human mononuclear leukocytes. In addition, the presence of hydrogen peroxide leads to increased control (no GSSG) levels of IL-6 and TNF-α. Besides that, being used in combination with hydrogen peroxide GSSG exerts a more pronounced (1.5- 2 fold) stimulatory effect on the

500 and 5000 μg/mL

The application of GSSG in 0.1% inosine solution and 0.1% cystamine solution results in a significant and dose-dependent increase of cytokine production, particularly with respect to IL-6 and TNFα (Tables 3 and 4).

Thus, the GSSG effect on the human peripheral blood mononuclear leukocytes *in vitro* manifests in considerable stimulation of the cytokine release into culture media, thereby confirming the stimulatory effect of GSSG on the natural cytokine-producing capacity of the human blood cells. The use of GSSG in combination with hydrogen peroxide, inosine, as well as cystamine results in a more prominent effect of GSSG in respect of induction of endogenous cytokine production.

Example #2

Effect of GSSG and its drug forms on cytokine and hemopoietic factor production as well as on hemopoiesis and immunity parameters in cyclophosphamide-induced hemo- and immunodepression.

Both oxidized (GSSG) and reduced (GSH) glutathione, as well as GSSG drug forms containing 0.003% hydrogen peroxide, or 0.1% inosine, or 0.1% cystamin, were evaluated in a murine model of hemo- and immunodepression induced by a single administration of cytostatic cyclophosphamide (CP).

The study was designed to evaluate the effect of a 5-day long administration of the test articles on the capability of the CP-treated murine splenocytes to produce IL-2 and GM-CSF *in vitro*. In addition, the number of blood leukocytes and lymphocytes and the bone marrow cellularity (number of karyocytes) were determined at 8 days after CP administration. Some animals receiving CP were then challenged with sheep red blood cells (SRBC), and the humoral immune response to the antigen was evaluated

Male CBA mice (18 to 20 g body weight) were given a single intraperitoneal injection [illegible]

[illegible]

- Control groups

- ◊ #1 — *intact animals* receiving a single injection of normal saline (NS) instead of CP injection, which further were treated with test article vehicle (normal saline);
- ◊ #2 — *control animals* receiving a single CP injection, which further were treated with test article vehicle (normal saline);
- ◊ #3 — animals receiving a single CP injection, which further were treated with a *reference article* (GSH dissolved in normal saline) in a dose of 5 mg/kg;

- Test groups:
  - ◊ #4 — animals receiving a single CP injection, which further were treated with the *test article* (GSSG dissolved in normal saline) in a dose of 5 mg/kg;
  - ◊ #5 — animals receiving a single CP injection, which further were treated with a variant of the *test article drug form* (GSSG dissolved in normal saline containing 0.003% H_2O_2) with a GSSG dose of 5 mg/kg;
  - ◊ #6 — animals receiving a single CP injection, which further were treated with a variant of the *test article drug form* (GSSG dissolved in normal saline containing 0.1% inosine) with a GSSG dose of 5 mg/kg,
  - ◊ #7 — animals receiving a single CP injection, which further were treated with a variant of the *test article drug form* (GSSG dissolved in normal saline containing 0.1% cystamine) with a GSSG dose of 5 mg/kg;

Twenty four hours after the CP injection, 5 animals in each group were immunized with SRBC (107 cells in 0.5 mL of NS, intraperitoneally)

On day 3 after the CP injection (24 hours after the immunization) the intraperitoneal

[illegible]

Twenty four hours after the completion of 5 day treatment course (on the 8th day after the CP injection) mice were euthanized and splenocyte cultures were aseptically

prepared for assessment of spontaneous production of IL-2 and GM-CSF by the spleen lymphocytes *in vitro*

Simultaneously, blood and marrow samples were collected for blood leukocyte and lymphocyte, and marrow nucleated cell counted

Serum samples from immunized animals were tested on level of SRBC agglutinins (the day 8 after the CP injection, and the day 7 after the immunization).

Table #5 shows the parameters of IL-2 and GM-CSF production by splenocytes, bone marrow and blood cellular indices, and the immune response to sheep red blood cells in mice receiving the test articles against the background of cyclophosphamide induced hemo- and immunodepression.

As is seen from the data, the use of both GSSG and GSSG solution in hydrogen peroxide brings IL-2 and GM-CSF splenocytic production almost back to normal whereas GSH shows no such effect. Also, both GSSG and its hydrogen peroxide solution exert a significant restorative effect on the bone marrow and blood parameters as well as immune response to SRBC.

Tables ## 6 and 7 give data on effects of pharmacologically active compositions containing GSSG (in combination with 0.1% of inosine, or 0.1% cyctamine) on tested parameter variations in mice with CP-induced hemo- and immunodepression. The findings show significant enhancing GSSG effects by inosine and cyctamine components with respect of IL-2b and GM-CSF production stimulation and restoration of bone marrow and blood cellularity. As it could be seen, GSH did not exhibit such stimulation. The maximum stimulation was achieved with the combination of GSSG and 0.1% inosine.

Thus, the use of the subject method in CP-induced hemo- and immunocompromised [illegible] production together with restoration of the bone marrow and blood cellular indices as well as immune response development to sheep red blood cells

Example #3

Effect of GSSG and its drug forms on cytokine and hemopoietic factor production as well as on hemopoiesis and immunity parameters in radiation-induced hemo- and immunodepression.

Both oxidized (GSSG) and reduced (GSH) glutathione, as well as GSSG drug forms containing 0.003% hydrogen peroxide, or 0.1% inosine, or 0.1% cystamine, were evaluated in a murine model of hemo- and immunodepression induced by a single irradiation in a total dose of 1 Gy.

The study was designed to evaluate efficacy of 7-day daily administration of the test articles (with the dosing started 2 hours post-exposure) on the capability of the splenocytes from mice exposed to radiation to produce IL-2 and GM-CSF *in vitro*. In addition, the number of blood leukocytes and lymphocytes and the spleen and bone marrow cellularity (number of karyocytes), as well as splenic and medullary colony-stimulating capacity, were determined at 8 days post-exposure.

Male CBA mice (18 to 20 g body weight) were irradiated with single dose of 180 kV X-rays filtered with 0.5 mm Cu (at 15 mA, distance — 70 cm, duration 2 min. and 28 sec.). The total absorbed dose comprised approximately 1 Gy.

Five groups of animals (with no less than 12 mice in each) were formed. Group description is represented below.

- Control groups:
  - ◊ #1 — *intact animals* receiving a sham irradiation procedure to reproduce a stress impact, which further were treated with test article vehicle (normal saline),
  - ◊ #2 — *control animals* irradiated in a dose of 1 Gy, which further were treated with test article vehicle (normal saline),
  - [illegible] with a *reference article* (GSH dissolved in normal saline) in a dose of 5 mg/kg,

- Test groups:
  - ◊ #4 -- animals irradiated in a dose of 1 Gy, which further were treated with the *test article* (GSSG dissolved in normal saline) in a dose of 5 mg/kg;
  - ◊ #5 — animals irradiated in a dose of 1 Gy, which further were treated with a variant of the *test article drug form* (GSSG dissolved in normal saline containing 0.003% H_2O_2) with a GSSG dose of 5 mg/kg.
  - ◊ #6 - animals irradiated in a dose of 1 Gy, which further were treated with GSSG in normal saline containing 0.1% inosine) with a GSSG dose of 5 mg/kg;
  - ◊ #7 - animals irradiated in a dose of 1 Gy, which further were treated with GSSG in normal saline containing 0.1% cystamine) with a GSSG dose of 5 mg/kg;

Two hours after the irradiation the intraperitoneal injections of the test or reference articles were started (as it has been described above). Injections were performed during 7 days: once a day, daily.

Twenty four hours after the completion of 7 day treatment course (on the 8[th] day after the irradiation), mice were euthanized and splenocyte cultures were aseptically prepared for assessment of spontaneous production of IL-2 and GM-CSF by the spleen lymphocytes *in vitro*.

Simultaneously, blood, spleen and marrow samples were collected for blood leukocyte and lymphocyte, and spleen and marrow nucleated cell counting.

Additionally hematopoietic colony formation ability of spleen and bone marrow cells was assessed by the method of direct count of colony forming units (CFU) in the spleens of irradiated CBA mice receiving intravenously spleen or bone marrow cells obtained from animals of control or test groups.

[illegible]

[illegible] stimulating capacity [illegible] bone marrow and spleen of the irradiated animals at 8 days post-exposure, are summarized in tables 8, 9, 10.

As is evident from the data of the tables, administration of GSSG, or its drug forms containing 0.003% hydrogen peroxide, or 0.1% inosine, or 0.1% cystamine, results in statistically significant recovery of IL-2 and GM-CSF production by splenocytes, whereas GSH produces no significant effect.

Furthermore, both GSSG alone and its pharmacologically active compositions exerted a significant normalizing effect on the blood, spleen, and bone marrow cellularity. In several instances the effect of GSSG dissolved in hydrogen peroxide has been found to be more prominent. For example, while GSSG *per se* exhibited no statistically significant effect (as compared to controls) on IL-2 splenocytic production, blood leukocytes, bone marrow cellularity, and bone marrow colonies, GSSG in hydrogen peroxide did produce a statistically meaningful effect. If compared with hydrogen peroxide, both inosine and cystamine were found to exert more prominent effect of enhancing the GSSG action, with the maximal effect being achieved in case of active composition of GSSG with inosine.

Thus, the use of the subject method in animals developed radiation-induced hemo- and immunodepression results in pronounced stimulation of the endogenous IL-2 and GM-CSF production, and also leads to an accelerated recovery of the cellular composition of the blood, lymphoid and hemopoietic organs as well as colony-forming activity of the bone marrow and spleen.

Example № 4.

Effect of GSSG and its drug forms on the process of proliferation and apoptosis of normal and tumor cells.

The ability of oxidized gluthation (GSSG), as well as its drug forms containing 0.003% hydrogen peroxide, or 0.1% inosine or 0.1% cystamine, to influence processes of cellular proliferation and/or death [illegible]

[illegible]

line HL-60 and normal human lymphocytes isolated form peripheral blood of healthy volunteers. Subsequent evaluation of the cell cycle parameters was carried out by the

flow cytofluorometry technique.

Venous blood of a healthy volonteers was collected into heparinized test-tubes which had been tested for endotoxine. A mononuclear fraction of blood leukocytes were obtained by centrifugation in gradient of fikoll-metrizoat (Histopaque, Sigma). Cell concentration was adjusted to $2x10^6$ cells per 1 ml of "complete" cell culture medium (RPMI 1640), containing 20 mM HEPES, 2 mM glutamine, 50 μg/mL gentamicine and 10% fetal calf serum. Cell viability was estimated by the Trypan blue exclusion method, then the cell suspension was placed into wells of 96-well microtiter plates - 200 000 cells per well.

Cells of HL-60 line were grown in RPMI-1640 medium with the addition of 10% fetal calf serum. Cultivation was carried out in closed flasks, the medium volume was 12 mL, it was changed every four days by centrifugation. The nature of the cells growth was suspensive. Evaluation of the test solution of GSSG (5000 μg/mL), as well as GSSG solutions containing 0.003% hydrogen peroxide, or 0.1% inosine, or 0.1% cystamine, was carried out using 6 cellular samples of normal lymphocytes and HL-60 cells for each test solution.

50 μL of each test solution were added to one or the other cell culture and thereafter cells were cultivated for 24-96 hours. Then, they were tested by the flow cytofluorometry to estimate DNA content in the cell nuclei. In case of apoptosis-like cellular death, the portion of cell nuclei with normal content of DNA became reduced, while the portion of cell nuclei containing abnormally small DNA quantity became larger.

The analysis procedure was following: after incubation completion, cells were centrifuged and transferred to a standard phosphate isotonic buffer pH 7.4, containing RNA-ase A (20μg/mL), ethidium bromide (fluorometric indicator for double stranded nucleic acid, 10 μg/mL) and $MgCl_2$ (5 mM). After that cells were disintegrated by nonionic detergent Triton X-100 (final concentration 0.1%). The suspension of cell nuclei thus obtained was analyzed by flow cytofluorometry with an argon laser as a source of light (wave length 488 nm). The red fluorescence due to DNA bound ethidium bromide was taken to be the measure of DNA content in the cell nuclei. In addition [illegible] morphology.

The study results are presented in Tables 11, 12 and Figure 1). The table 11 shows the

presence of GSSG or its drug forms promoted proliferation of normal lymphocytes of healthy volonteers, which resulted in increase in their number, while flow cytofluorometry analysis did not reveal any changes characteristic for apoptosis-like cell death (Figure 1c-d).

Observation carried out on cell cultures of the tumor cells of myeloid line HL-60 revealed ability of GSSG (as well as its drug forms) to slowdown the proliferation of transformed cells. Table 12 shows that GSSG compositions with hydrogen peroxide, inosine and cystamine inhibit cell HL-60 proliferation better than GSSG alone. The flow cytofluorometry analysis demonstrates the slowdown of cell growth of the HL-60 line cells was associated with characteristic morphological indications of apoptosis-like death: sphere-like cells became multi-fragmented with plural interceptions, the number of cell nuclei with normal content of DNA fell down, while there was an increase in portion of nuclei with abnormally low DNA content (Fig. 1a - 1b).

Thus, the results obtained enable to declare the dual functional properties of GSSG and its drug forms which selectively induce proliferation slowdown and apoptosis-like death of tumor cells while accelerate proliferation of normal human cells (lymphocytes) without any signs of their apoptosis. The application of GSSG in combination with inosine produces the most prominent effect of GSSG in respect of normal cells.

Figure 1.

Research of apoptosis-induced preparation activity in cultivated mammal cells.

Figure 1a

Cytofluorimetry analisis of cells HL-60

Figure 1b

Cytofluorimetry analisis of cells HL-60 in the presence of the preparation

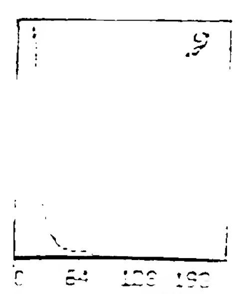


Figure 1c

Cytofluorimetry analisis of human lymphocytes

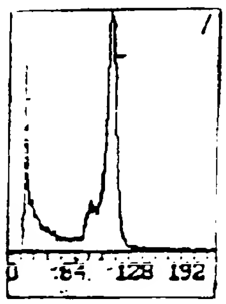
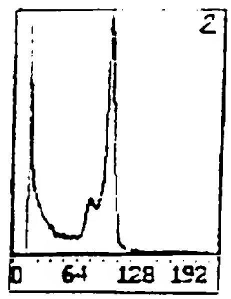


Figure 1d

Cytofluorimetry analisis of human lymphocytes in the presence of the preparation



Example № 5.

Effect of GSSG and its drug forms on progression of experimental tumors in mice.

An antitumor activity of GSSG, as well as its drug forms containing 0.003% hydrogen peroxide or 0.1% inosine, or 0.1% cystamine, was evaluated in the two mouse models of the tumor process induced by the intraperitoneal inoculation of leukemia P388 and leukemia L1210 cells. An influence of 7 day course of test article daily administration was studied in respect of variations of serum cytokine levels (IL-I, IL-2, IL-6, IFNα, TNF). In parallel, the tumor progression was estimated using the two integral indices: pace of mouse weight gain due to accumulation of ascitic fluid, and by animal mean survival time after inoculation.

The study was carried out on DBA/2 mice weighing 18-21 g. First, tumor cell passage was performed using 6 animals for each cell line. For this, cells kept at the temperature of the liquid nitrogen were de-frozen and adjusted to the concentration of $5x10^6$ cells/mL by sterile Hanks' solution. Then, 6 mice were intraperitoneally inoculated

Ascitic fluid was collected 6 days after the inoculation with L1210 cells and 8 days after the inoculation with P388 ones. Thus obtained, the samples of passaged tumor

cells were used for the main experiments. The fluid liquid was dissolved by sterile Hanks' solution so that cell concentration be $5x10^6$ cells/mL for P388 cells and $5x10^5$ cells/mL for L1210 cells.

Nine groups of animals with no less than 15 mice each were formed for experiments with either tumor cell line. Mice were inoculated with 0.2 mL of resultant cell suspensions per mouse (10^6 P388 cells/mouse, and 10^5 L1210 cells/mouse). 24 hours after the tumor cells inoculation, animals were given the first injections of the test articles or vehicles. The test article injections were made daily till the 14[th] day of the experiment or till the animal death. The volume of solutions injected comprised 0.01 mL/g body weight.

Description of nine groups of animals formed for experiments with either tumor cell line is given below.

- Control groups:
    - ◊ #1 - *intact animals*, receiving imitation of tumor cell inoculation (injection of normal saline) which further were treated with normal saline throughout the entire experiment;
    - ◊ #2 - *control animals*, inoculated with tumor cells, which further were treated with test article vehicle (normal saline);
- Control groups:
    - ◊ #3 - experimental animals, inoculated with tumor cells, which further were treated with test article (GSSG dissolved in normal saline) in a dose of 5 mg/kg;
    - ◊ #4 - experimental animals, inoculated with tumor cells, which further were treated with a variant of test article drug form (GSSG dissolved in normal saline containing 0.003% of hydrogen peroxide), with a GSSG dose of 5 mg/kg;
    - ◊ #5 - experimental animals, inoculated with tumor cells, which further [illegible] in normal saline containing [illegible] of inosine), with a GSSG dose of 5 mg/kg,

◊ #6 - experimental animals, inoculated with tumor cells, which further were treated with a variant of test article drug form (GSSG dissolved in normal saline containing 0.1% cystamine), with a GSSG dose of 5 mg/kg,

◊ #7 - experimental animals, inoculated with tumor cells, which further were treated with a variant of drug form component (normal saline containing 0.003% of hydrogen peroxide), without GSSG;

◊ #8 - experimental animals, inoculated with tumor cells, which further were treated with a variant of drug form component (normal saline containing 0.1% of inosine), without GSSG;

◊ #9 - experimental animals, inoculated with tumor cells, which further were treated with a variant of drug form component (normal saline containing 0.1% of cystamine), without GSSG;

Tables 13 and 14 contain results on test article efficacy evaluation as to variations of cytokine endogenous production, as well as data on integral parameters of the tumor process progression. The results obtained show that both GSSG and its drug forms have a substantial cytokine inducing effect, reliably retard (if compared to the control groups) the accumulation of ascitic fluid and increase the mean survival time. GSSG alone and GSSG together with 0.003% of hydrogen peroxide increase more remarkably the IL-1 and IFNα serum levels, whereas GSSG in combination with 0.1% inosine and 0.1% cystamine cause a larger increase in IL-2, IL-6, TNFα serum levels

The most prominent antitumor effect in respect to slowdown of ascitic fluid accumulation and prolongation of the mean survival time for either tumor models (P388 and L1210 leukemia) were obtained with GSSG in combination with 0.1% cystamine.

[illegible]

[illegible] inhibition of progression of experimental tumors and prolongation of the mean survival time

New properties of a previously known substance - oxidized glutathione (GSSG), and its pharmacologically active compositions, containing 0.003% hydrogen peroxide, or 0.1% of inosine, or 0.1% cystamine, found in the pre-clinical studies, are thought to be sufficient to declare that GSSG and its pharmacological formulations possess an obvious biological and pharmacological activity, as well as a therapeutic effect. This justifies the application of the corresponding drug forms of GSSG alone and GSSG in combination with pharmaceutically acceptable components capable of extending the oxidized glutathione half life, for preventing and treating the diseases in which stimulation of endogenous production of cytokines and hemopoietic factors is advantageous and considered beneficial by those who skilled in the art.

The following examples (## 6–12) of the GSSG drug forms clinical use support the idea of utilizing GSSG as an inducer of the endogenous cytokine and hemopoietic factor production in man, and provide for the method for disease treatment based on the above GSSG properties.

Example #6

Effect of GSSG drug form on the endogenous cytokine and erythropoietin production in patients having neoplastic disease

Data presented in this example demonstrate the GSSG stimulatory effect on the endogenous cytokine and hemopoietic factor production in cancer patients. GSSG solution (5 mg/mL) was administered intravenously, slowly, every other day at 5 mg per injection. The cytokine endogenous production was determined by their blood levels prior to the first administration (with blood collected 24 hours before dosing) and after the third and the seventh injections The cytokine levels were assessed by immunoenzyme technique using commercially available kits (Medgenix, Belgium), and

As seen from the data given in Table 15, a pronounced stimulation of the endogenous cytokine (IL-1β, IL-6, TNF-α, IFN-α) and erythropoietin was noted as soon as after

three first injections of GSSG. After the seventh administration (14 days of treatment) a manifold increase in the cytokines and erythropoietin blood levels was observed in the majority of cases.

Example #7

Stimulation of the endogenous cytokine and erythropoietin production in a patient suffering from colorectal cancer complicated with chemotherapy-induced hemodepression

A 44-year old female patient was operated for colorectal mass grown through the ovary and metastases in the mesenteric and omental lymph nodes ($T_4N_3M_1$). Postoperatively, 5-fluorouracil chemotherapy was conducted (total course dose 5.5 g) with resultant severe hemotoxicity.

One month after the chemotherapy the patient was reexamined, and ultrasonography of the peritoneum and computed tomography of the liver revealed an oval-shaped 13 × 10 mm solitary metastasis in the left liver lobe. Repeat blood counts showed incomplete recovery of the blood indices (leukopenia, lymphopenia, anemia, and thrombocytopenia of various severity were noted) rendering further chemotherapy impossible.

Laboratory parameters prior to the use of the oxidized glutathione drug form (5 mg of GSSG in 1 mL of 0.003% hydrogen peroxide) are listed in Table 16. The treatment according to the subject method was commenced with GSSG given intravenously for seven days, 5 mg once daily. After a 3-day interval, the treatment was resumed with 15 mg daily dose, IV, 10 days. This course was followed by a 7-day recess after which the therapy was continued with GSSG being given every other day IM, 15mg daily (a total of 20 injections).

[illegible]

[illegible]asonography of the peritoneum and computed tomography of the liver showed a considerable shrinkage (more than 50% of the pretreatment size) of the solitary hepatic metastasis. The post-treatment immunological indices are given in Table 16.

As seen from the data, both red and white blood cell counts have significantly improved, platelets almost completely recovered, ESR reduced, $CD4^+$, $CD8^+$, NK cell numbers increased. A considerable stimulation of the endogenous cytokine and erythropoietin production, with TNF (together with increased natural killers) being probably responsible for the regression of the hepatic metastasis. These changes were accompanied by an improved general condition of the patient.

This clinical case indicates apparent therapeutic efficacy of the subject method. The administered therapy resulted in significant stimulation of the endogenous cytokine and hemopoietic factor production, reduction in hepatic metastasis size, normalization of immunity parameters, and overall improvement in the patient's wellness.

Example #8

Stimulation of the endogenous cytokine production in an AIDS patient with cryptococcal meningitis

A 28-year old male was admitted with a previously confirmed diagnosis of AIDS, stage 3/4C (WHO staging system) in moderately grave condition. The patient presented with paroxysmal headache, dizziness, and vomiting. Weight 47 kg, Karnofsky score 60, torpid, fevers up to 39°C, dyspnea at rest. Neurological examination revealed nuchal rigidity and diminished knee, ankle, biceps and triceps reflexes. Cerebrospinal fluid culture was positive for *Cryptococcus neoformans* which served the basis for making the diagnosis of cryptococcal meningoencephalitis, and the AIDS stage was refined as 4C.

A vigorous infusion therapy was started. In addition to palliative therapy the patient received a course of Fungizone (Amphotericin B) with no positive outcome. The neurologic symptomatology and the patient's general state continued to deteriorate. A low to moderate grade fever (37.5–38.5°C) persisted.

[illegible] oxidized glutathione was started ([illegible] mg [illegible]). The patient had a significant drop in $CD4^+$ and $CD8^+$ peripheral blood counts as well as anemia and overall lymphopenia (see Table 17).

The patient received the treatment according to the subject method for 3 months (1 mL of the GSSG solution per administration). During the first month of treatment the patient was dosed every other day (first 10 days intravenously, the rest of the month — intramuscularly); during the second month the patient received the drug every three days (first 10 days IV, the rest of the month — subcutaneously).

By the middle of the first month therapy, the patient's condition improved significantly with the neurologic sign alleviated and low-grade fever not exceeding 37.5°C. In the course of treatment, the patient's cerebrospinal fluid was mycologically examined twice (cytology, cultures, latex-agglutination test for cryptococcal antigen). Towards the end of the first month therapy the number of viable *Cryptococcus neoformans* organisms was found to be considerably reduced. By the end of the second month the cytological, culture, and immunologic tests showed cerebrospinal fluid to be free of the pathogen. Because of the drastic improvement in the patient's state, during the third month the drug was given once weekly IM.

The hematology/immunology findings upon the therapy completion are given in Table 7. As evident from the table, the anemia signs have reduced and a significant increase in the number of lymphocytes and their subsets has taken place. These findings constitute AIDS restaging from 4C to 4B.

Noteworthy is the sizable elevation of the cytokine blood levels, with IL-2, IL-6, and IFN-γ playing the key role in the host defense against pathogenic fungi

At discharge, the patient's condition was found satisfactory with body weight being 60 kg (weight gain made up 21.7% of the admission), normal body temperature, Karnofsky score of 90, and no neurological signs.

Example #9

Stimulation of the endogenous cytokine production and therapeutic eff[illegible]

[illegible] immunodeficiency [illegible]

A 38-year old male had been observed for 2 years with the diagnosis of AIDS, stage

3C (WHO Staging System). During the preceding year, recurrent episodes of oral and esophageal candidiasis had been recorded as well as chronic intestinal isosporiasis manifested by poor appetite, nausea, frequent vomiting and watery stools containing blood and mucus. Repeatedly used cotrimoxazole (trimethoprim plus sulfamethoxazole, TMP-SMX) had produced unsteady remissions with rapid recurrence of the symptomatology. During the last month prior to admission another relapse of isosporiasis had occurred. The treatment with cotrimoxazole, emodium (loperamide) had brought no relief. The patient's condition had been gradually deteriorating: refractory fever 38°C and above, 6–7 loose bloody and mucous stools a day, vomiting, advancing weight loss (15% of the premorbid weight in one year). The patient had been admitted with progressive worsening of his condition.

On admission, the patient presented with moderately grave condition, Karnofsky score of 50, fever 38.2°C, emaciation (body weight 42 kg), virtually total lack of subcutaneous fat, pallor of skin, the signs of oral and esophageal candidiasis. Stool examination revealed a large number of *Isospora belli* oocysts.

By the time the therapy according to the subject method was started, the patient had lymphopenia, marked decline in $CD4^+$ and $CD8^+$ lymphocytes, hypoproteinemia (see Table 18).

The patient received the oxidized glutathione drug form (5 mg of GSSG in 1 mL 0.003% hydrogen peroxide) for 2 months (1 mL of the GSSG solution per administration). During the first month of treatment the patient was dosed every other day (first 10 days intravenously, the rest of the month — intramuscularly); during the second month the patient received the drug every three days (first 10 days IV, the rest of the month — subcutaneously).

The patient's condition began to noticeably improve after the first two weeks of

[illegible] times a day with stools being blood-free [illegible] body temperature only occasionally exceeded 37°C. At the end of the second month stool reexamination showed feces to be negative for *Isospora belli*. Because of the drastic improvement in the patient's

state, during the third month the drug was given prophylactically once weekly IM. No relapses of the disease were noted.

The findings of hematology/blood chemistry evaluations upon the therapy completion are given in Table 18. As seen from the table, hypoproteinemia has reduced, the number of lymphocytes and their subsets considerably increased with the resultant restaging of AIDS to 3B stage according to the WHO Staging System.

Noteworthy is the marked increase of the cytokine blood levels, with IL-2 and IFN-γ known to play an important part in the host defense against protozoan infections.

As a result of the therapy administered the patient's condition improved drastically, fatigue alleviated, appetite regained. The weight gain comprised 30 % of the admission value, Karnofsky score — 90. On physical examination the patient's condition was rated as satisfactory. During 1.5 month follow-up no diarrhea relapses were reported.

Example #10

Stimulation of the endogenous erythropoietin production and therapeutic effect in patient with hypoplastic anemia and pancytopenia

A 37-year old male had been observed for about a year with anemia of unknown origin the severity of which had been gradually building up. For 10 months he had been troubled with fatigability, dizziness, frequent nasal bleedings, unusual susceptibility to respiratory infections, three episodes of pneumonia with one of them being the croupous pneumonia. During the year the patient had lost 10 % of his usual weight. Repeated outpatient treatment with oral and intravenous iron preparations, folic acid, B vitamins, including B_{12}, had produced no effect.

On admission the patient presented [illegible] moderate [illegible] and isolated petechial [illegible] analyses have revealed moderately severe to severe fairly hypochromic (color index 0.7–0.9) normocytic anemia (1.5–2.5 × 10^{12}/L), anisocytosis and poikilocytosis.

moderate leukopenia, and thrombocytopenia within 50-80×10^9/L.

An aggressive infusion therapy with iron preparations, folic acid, cyanocobalamin, vitamins, prednisone, and repeated erythrocyte transfusions resulted in only marginal relief.

Bone marrow differential (punch biopsy) revealed marked hypocellularity with medullary cavities populated predominantly with fat cells. Both myeloid and erythroid lineages are significantly suppressed with the erythroid/myeloid ration noticeably diminished. Megakaryocytes are scant in number with relative increase in nondifferentiated cells, plasma cells, and blasts. Iron stores are enriched. Diagnosis: hypoplastic anemia of unknown origin, pancytopenia.

Complete blood count and erythropoietin levels by the time the oxidized glutathione drug form (5 mg GSSG in 1 mL of 0.003% hydrogen peroxide) was started are given in Table 19. As is evident from the table, the laboratory findings are consistent with those characteristic of hypoplastic anemia with no typical increase of erythropoietin blood level, however. Moreover, the erythropoietin level was found to be considerably below the lower normal limit (9.2 pg/mL with the reference range 30–170 pg/mL corresponding to 3–17 mIU/mL).

The oxidized glutathione formulation therapy was started with intramuscular injections of 1 mg GSSG b.i.d for three days. Further the dose was escalated up to 5 mg b.i.d. for 7 days. Blood counts have shown less severe anemia. From that point, the drug form was dosed at 10 mg IM once daily for 10 days and then, with RBC counts steadily recovering, the therapy was switched to IV administration of GSSG, once every three days for 30 days. Vitamins and iron preparations were given concomitantly *per os*.

The hematology findings and erythropoietin levels obtained 50 days following the [illegible] treatment [illegible] listed [illegible] from the table [illegible] RBC and WBC counts significantly improved, as did the platelet counts, ESR reduced, erythropoietin levels exceeded the upper normal limit. Clinically, fatigue, dizziness,

and dyspnea disappeared. On examination, no petechial spots or bruises could be found with no nasal bleedings observed or reported. The weight gain made up 5.5 kg (8% of the premorbid weight).

Bone marrow reexamination (punch biopsy upon therapy completion) found the myeloid tissue to occupy 60% of the medullary cavities with erythroid/myeloid ratio in the myeloid tissue isles exceeding the norm. There were normoblastoid hyperplasia signs with megaloblastoid cells found in normoblast congregations. Mast cells were encountered, megakaryocytes were present in abundance. Iron stores appeared to be somewhat enriched.

This clinical case indicates a clear therapeutic efficacy of the drug form. Due to the treatment administered the initially suppressed endogenous erythropoietin production received a potent boost. As a result, the hematology parameters virtually recovered and the anemia clinical sings resolved. The patient was discharged in satisfactory condition.

Example #11

Stimulation of endogenous cytokine production and the therapeutic effect in a patient with a stomach cancer, peritonal matastases, ascites, splenomegaly and cholestatic hepatitis

A 33year old patient was diagnosed as having stomach neoplasm for more than 2 years (adenocarcinoma of moderate differentiation degree). In 1993 the patient was operated for malignant stomach ulcer and numerous dense lymph nodes were found in the *porte hepatis* which were considered to be metastases.

In January 1994 the course of chemotherapy (5FU) was complicated by the severe cholestasis and percutaneus drainage of the left and right liver ducts was undertaken.

[illegible]

[illegible]ansver drains with Brown's anastomosis

In November 1995 the state of the patient worsened. According to the examinations

the patient experienced an active secondary hepatitis. The liver was enlarged and painful and protruded from the costal arch up to 5-6 sm. Blood chemistry indices proved to be persistently abnormal: bilirubin - 40.0 due to indirect (up to 31.0); activity of amino transferases - approximately 6 times higher than upper normal limit, hypoalbuminemia was up to 26%; and there was also hypergammaglobulinemia; hypercholesterolemia was up to 10.2 μmol/l.

During fibrogastroscopy (November, 1995) tumor of stomach located in the middle area of the stomach body and extended about 8 cm was confirmed. The tumor was solid-like. Stomach walls were rigid. Histology examination defined the tumor as adenocarcinoma of moderate degree differentiation. In December, 1995 the patient was exposed to explorative laparotomy. Ascites was found with plural metastases all over the peritoneum, splenomegaly. The patient was identified as unoperable.

A decision was taken to apply GSSG drug form containing 0.1% inosine. The drug was injected parenterally (intramuscular and intravenous), and additionally, the drug form was used via local injections around the tumor tissue with the help of endoscope. An average doses which were used for intramuscular and intravenous injections - 0.1-0.5 mg/kg, and for local injections - up to 50 mg *in situ*. Parenteral injections of the drug were applied every other day, b.i.d. (intravenous injections at the morning, and intramuscular ones - at the evening), during three weeks, and after that - two times in a week, during four weeks. Two months after the beginning of the treatment with the drug form used fibrogastroduodenoscopy showed that esophagus was passable, mucous membrane was pink, cardia rosette was partly closed. On empty stomach moderate amount of foamy secret was in the stomach, which was intensively colored with bile. The tumor extent was 5 cm. At the same time, substantial improvement of hematology and blood chemistry indices was found.

Four month after, the liver protruded 1 cm beyond the rib arch. On palpation the li... [illegible] ... some [illegible] examination showed the appearance [illegible] instead on the place of some areas previously affected with tumor tissue. Fibrogastroduodenoscopy performed in May, 1996, showed that the esophagus was

partly closed. There was light turbid liquid in the stomach, which contained saliva. Mucous membrane was pink. The tumor was 3.6 cm in extent with the stomach walls being elastic. Duodenum was passable.

By comparison with results of examination conducted before treatment with the use of the GSSG drug form mentioned (November, 1995) the tumor was shrunk in its extent for 55%. Simultaneously there were significant beneficial changes in hepatic tests, hematology and immunology indices (see table 20).

Thus, the treatment according to the present invention resulted in partial regress of neoplastic process with simultaneous obvious beneficial changes in hematology, blood chemistry and immunology parameters, and significant improvement of life quality.

Example #12

Stimulation of endogenous cytokine production and the therapeutic effect in a patient with skin cancer (Merkel's cell carcinoma), local lymph node metastases and chemotherapy-induced hemo- and immunodepression.

A male patient, 64 years old, has been under medical supervision since August, 1995 when a hyperemic painless mass appeared in scapular area, which progressively grown in size. After a month time the mass spread over the axillary space, kept increasing, and became painful. A fever appeared (38.9° C. Hystological and immunological examination in October, 1995 made the diagnosis clear: neuroendocrinal form of skin cancer (Merkel's cell carcinoma) stage III

In December, 1995 the patient was given a course of CMF chemotherapy (cyclophosphamide+ methotrexat+fluorouracil) without appreciable curative effect. At the same time an obvious hemopoiesis depression (leukocytes 2.4x10^9/L) developed with simultaneous growth of cervical and superclavicular lymph nodes associated with [illegible] hyperemia

In January - February, 1996 chemoterapy scheme was changed cysplatine + cyclophosphamide (CP instead of CMF). The chemotherapy brought about the

following complications - cytopenia (leukocytes - 1.4 x 10^9/l), cardiotoxicity in the form of ischemia deterioration. After the 2nd course of chemotherapy a substantial tumor progression was observed: necrosis in the left subaxillary area with fistula formation; edema of the left arm; infiltrating growth into soft tissues in the area of the left shoulder and the left subaxillary tissues; intoxication; persistent fever (38.8°C) Because of inefficacy of chemotherapy and the obvious progression of the process, it was decided to administer a course of GSSG drug form in combination with 0.1% cystamine, together with chemotherapy (CMF).

After 10 daily injections of the GSSG drug form used (intravenously and intramuscularly, the dose 0.1-0.5 mg/kg per an injection), it was noticed: the following changes in the patient's status was revealed: improved quality of life (good appetite, mobility); ulceration drying out, abolition of suppurative discharge; fistula scarring, 30% tumor shrinkage; normal body temperature; limitation of hyperemic areas , the improvement of hematology indices.

The 3rd and the 4th courses of chemotherapy (CMF) were carried out together with GSSG drug form (intravenous and intramuscular injections, b.i.d., intravenous dose 0.5 mg/kg; and intramuscular dose 0.2 mg/kg). Parenteral administration of the preparation was 3 times in a week, with local injections in the two spots around the tumor through the endoscope once a week (up to 25 mg for each spot). The following results was obtained: tumor process regression; good endurance of chemotherapy, the disappearance of pain syndrom, constant improvement of life quality, restoration of immunity and hemopoiesis, increasing level of cytokines and hemopoietic factors (see table 21).

In two months of the tretment with the use of the present invention there was a stable level of endogenous production of cytokines and hemopoietic factors; the diminution of the left cervical and supraclavicular lymph nodes, the 70% shrinkage of tumor size [illegible] depression.

The clinical observation proves the clear curative effect of the the treatment according

to the present invention: together with the obvious stimulation of endogenous production of cytokines and hemopoieticfactors there were a substantial decrease in tumor size, improvement of life quality, and beneficial changes in hematology, blood chemistry and immunology parameters.

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What is Claimed is:

1 A method of utilizing oxidized glutathione - a dimer of reduced glutathione, a tripeptide with structure γ-glutamyl-cysteinyl-glycine, where two molecules of the tripeptide are linked via covalent disulfide bond between the cysteine residues - as a stimulator of endogenous production of cytokine and hemopoietic factors for preparation of pharmaceutical drugs for treating neoplastic, infectious, hematologic, immunologic and other diseases in which stimulation of the endogenous cytokine and hemopoietic factor production is considered beneficial.

2 A therapeutic agent for treating neoplastic, infectious, hematologic, immunologic and other diseases in which stimulation of the endogenous cytokine and hemopoietic factor production is considered beneficial, containing an effective amount of oxidized glutathione, dimer of reduced glutathione (tripeptide, γ-glutamil-cysteinyl-glycine) where two molecules of the tripeptide are linked via covalent disulfide bond between the cysteine residues, as an active substance.

3. A drug formulation of the therapeutic agent of claim 2 wherein said substance is formulated in the form of injectable solution of oxidized glutathione in pharmaceutically acceptable solvent

4 A drug formulation of the therapeutic agent of claim 2 wherein a pharmaceutically acceptable component capable of enhancing and prolonging the therapeutic effect via increasing the half-life of oxidized glutathione introduced into biological media.

5 A drug formulation of claim [illegible] peroxide [illegible] peroxide solution

6 A drug formulation of claim 4 wherein said drug form contains inosine (hypoxanthie-9-D-ribofuranoside) as a pharmaceutically acceptable component, including inosine solution.

7 A drug formulation of claim 4 wherein said drug form contains cystamine (2,2'-Dithio-bis[ethylamine]) as a pharmaceutically acceptable component, including cystamine solution.

8 A method of enhancing and prolonging the ability of oxidized glutathione to stimulate endogenous production of cytokine and hemopoietic factor wherein oxidized glutathione is used in a pharmaceutical composition containing a pharmaceutically acceptable components able to prolong the half-life of exogenous oxidized glutathione introduced in oxidized form into biological media, such as donors of reactive oxygen intermediates (for instance hydrogen peroxide and other prooxidant active compounds), or hypoxanthine derivatives, (for instance hypoxanthine riboside and other nucleoside derivatives of inosine), or reversible inhibitors of pentose phosphate pathway of glucose oxidation (for instance cystamine).

9 A method of treating neoplastic, infectious, hematologic, immunologic and other diseases in which stimulation of the endogenous cytokine and hemopoietic factor production is considered beneficial, including parenteral administration of drug formulations of claims 3, 4, 5, 6 and 7 in therapeutically effective doses per 1 kg of body weight or per 1 m^2 of the body surface, and further comprising one or more administrations of said drug forms to a subject in need thereof in pulses of one or more days or continuously until the therapeutic effect is achieved

CYTOKINE AND HEMOPOIETIC FACTOR ENDOGENOUS PRODUCTION ENHANCER AND METHODS OF USE THEREOF

Table 1. GSSG effect on in vitro cytokine production by human mononuclear leukocytes. (M±m)

GSSG (μg/mL)	Cytokine production (pg/mL)			
	IL-1β	IL-6	TNFa	IFNa
5000	259±36.8*	2518±264*	1900±206*	511±64.1*
500	275±39.3*	2113±132*	1525±163*	514±56.2*
50	202±24.9*	1910±205*	813±90.8*	407±51.4*
5.0	88.5±13.5*	550±61.3*	314±44.7*	109±12.1
0.5	56.0±9.1	430*±55.6	99.1±11.6	130±14.9
Control (RPMI)	46.0±6.8	129±12.4	88.7±9.3	98.3±14.0

(*) — differences are statistically significant ($p < 0.01$) as compared to the control.

Table 2. Effect of GSSG in combination with 0.003% hydrogen peroxide on *in vitro* cytokine production by human mononuclear leukocytes. (M±m)

GSSG (μg/mL)	Cytokine production (pg/mL)			
	IL-1β	IL-6	TNFα	IFNα
5000	720±81.3*	4035±518*	2640±355*	849±102*
500	650±67.1*	4007±419*	2100±294*	905±141*
50	511±55.1*	3859±425*	1308±164*	468±69.3*
5.0	212±31.7*	1680±207*	502±86.4	160±37.0
0.5	63.0±7.8	851±111	318±47.8	98.3±18.7
[illegible] RPM + [illegible]003 H_2O_2	[illegible]	[illegible]	[illegible]	[illegible]

(*) — differences are statistically significant ($p < 0.01$) as compared to the control

Table 3. Effect of GSSG in combination with 0.1% inosine on *in vitro* cytokine production by human mononuclear leukocytes. (M±m)

GSSG (μg/ml)	Cytokine production (pg/mL)			
	IL-1β	IL-6	TNFa	IFNa
5000	665±73,5*	5720±498*	5900±317*	1010*±160,5*
500	790±68,85*	3840±352*	4520±*366	1318±152*
50	416±44,0*	4910±205*	1869±90,8*	311±51,4*
5.0	205,8±18,3*	2680±196*	765±67,1*	117±10.4*
0.5	183±20,0*	1505±138*	597±48,6*	66,3±7,8*
Control (RPMI+0.003% H_2O_2)	60,9±5,59*	131±11,7*	83,5±9,6*	89,5±10.0*

(*) — differences are statistically significant ($p < 0.01$) as compared to the control.

Table 4. Table 4 Effect of GSSG in combination with 0.1% cystamine on *in vitro* cytokine production by human mononuclear leukocytes. (M±m)

GSSG (μg/ml)	Cytokine production (pg/mL)			
	IL-1β	IL-6	TNFa	IFNa
5000	810±75,36*	4910±503*	5140±466*	1060±799*
500	540±60,03*	4000±307*	3800±307*	780±180,3*
50	490±45,5*	3800±3183*	2600±183	460±39*
5.0	316±30,5*	2610±207*	1408±101*	100±17,7*
0.5	155±9,7*	10±110*	709±67,3*	107,6±8,13*
Control (RPMI + 0,1% [illegible]	60,8±6,55*	65,4±77.0*	377±28,9*	114±10.01*

[illegible]ifferences are statistically significant (p [illegible]) as compared to the contr[illegible]

Table 5. Effect of the test articles on IL-2 and GM-CSF production by splenocytes, bone marrow and blood cellular indices, and immune response to SRBC in cyclophosphamide treated mice. (M±m)

Parameter	n	Intact animals	Cyclophosphamide-treated animals			
		Normal saline	Normal saline	GSH	GSSG	GSSG + H_2O_2
IL-2 production by splenocytes, (U/mL)	10	39.7±5.4	11.1±3.0*	17.2±3.5*	28.1±3.9#@	34.7±5.1#@
GM-CSF production by splenocytes, (colonies /10^5 cells)	10	180.0±14.2	34.3±9.1*	58.2±7.2*	129.1±13.4#@	170.1±16.9#@
Blood leukocyte count, 10^9/L	10	11.9±1.81	4.7±1.25*	5.2±1.36*	8.5±0.81#@	9.4±1.40#@
Blood lymphocyte count, 10^9/L	10	7.4±0.85	3.1±0.56*	4.3±1.13*	6.2±1.28#	6.8±1.04#
Bone marrow nucleated cell number, 10^6/L	10	53.7±8.7	23.8±5.0*	32.2±4.4*	45.4±3.9#@	52.3±4.7#@
SRBC agglutinin titer ($\log_2$)	5	5.33±0.74	1.47±0.35*	1.94±0.34*	3.68±0.59*#	4.12±0.37*#

Differences are statistically significant ($p < 0.05$) as compared

(*) — to the group of intact animals, (#) — to the control group (CP + normal saline),

(@) — to the group of animals treated with GSH.

Effect of GSSG in combination with 0.1% inosine on IL-2 and GM-CSF production by splenocytes, bone marrow and blood cellular indices, and immune response to SRBC in cyclophosphamide treated mice. (M+m)

arameter	n	Intact animals	Cyclophosphamide-treated animals			
		Normal saline	Normal saline	GSH	GSSG	GSSG + 0,1% inosine
production by ocytes, (U/mL)	10	34,4±4,2	9,2±1,9*	15,3±2,7*	29,8±3,158#@	39,7±4,8#@
F production by lenocytes, nies /10^5 cells)	10	168,0±14,9	25,5±4,2*	63,4±7,8*	143±15.06#@	196,3±16,6#@
Bl ocyte count, 10^9/L	10	12,3±1,4	5,03±0,85*	6,3±0,05*	9,5±1,01#@	10,1±1,36#@
B mphocyte count, 10^9/L	10	8,2±0,09	2,8±0,67*	4,6±0,78*	6,7+0,81#	7,18±0,74#
B row nucleated cell nber, 10^6/L	10	61,3±8,05	19,7±2,9*	36,4±4,5*	48,99±5,14#@	69,4±17,7#@
SR gglutinin titer ($\log_2$)	5	6,03±0,71	1,05±0,28*	1,62±0,27*	4,08±0,58*#	5,13±053*#

Diff are statistically significant ($p < 0.05$) as compared:

(* group of intact animals; (#) — to the control group (CP + normal saline);

(@ group of animals treated with GSH.

Tat ffect of GSSG in combination with 0.1% cystamine on IL-2 and GM-CSF production by splenocytes, bone marrow and blood cellular indices, and immune response to SRBC in cyclophosphamide treated mice. (M±m)

arameter	n	Intact animals	Cyclophosphamide-treated animals			
		Normal saline	Normal saline	GSH	GSSG	GSSG +0.1% cystamine
production by cytes, (U/mL)	10	43,5±4,01	14,0±2,7*	20,3±2,6*	30,9±3,03#@	38,8±4,53#@
G production by plenocytes, ies /10^5 cells)	10	190,5±18,4	42,0±5,7*	66,7±7,8*	137,0±13,09#@	183,7±17,8#@
B ukocyte count, 10^9/L	10	12,3±1,28	4,95±0,88*	6,2±1,06*	7,8±0,84#@	10,5±1,56#@
Bl nphocyte count, 10^9/L	10	8,2±0,72	3,6±0,63*	5,31±0,77*	7,2±0,96#	7,8±0,84#
B arrow nucleated mber, 10^6/L	10	61,3±5,9	28,5±4,2*	36,4±4,5*	48,9±5,14#@	56,7+4,91#@
SRB glutinin titer (log_2)	5	6,03±0,60	1,78±0,36*	2,09±0,37*	4,08±0,57*#	4,29+0,41*#

Di are statistically significant ($p < 0.05$) as compared:

(*) group of intact animals; (#) — to the control group (CP + normal saline);

(@ group of animals treated with GSH.

Table 8. Effect of the test articles on IL-2 and GM-CSF production by splenocytes, bone marrow, spleen and blood cellular indices, and bone marrow and spleen hematopoietic colony formation capability in irradiated mice. (M±m)

Parameter	n	Sham-irradiated animals	Irradiated animals			
		Normal saline	Normal saline	GSH	GSSG	GSSG + H_2O_2
IL-2 production by splenocytes, (U/mL)	12	41.2±4.4	5.0±0.5*	8.6±1.3*	25.1±4.9*#@	37.1±3.4#@
GM-CSF production by splenocytes, (colonies /10^5 cells)	12	120.2±12.4	20.7±8.6*	31.8±3.9*	93.1±11.5#@	106.4±5.2#@
Blood leukocyte count, 10^9/L	12	12.7±1.3	3.4±0.9*	4.8±0.8*	8.7±1.3*#@	10.7±2.0#@
Blood lymphocyte count, 10^9/L	12	7.9±0.7	2.2±1.3*	3.4±0.6*	5.9±0.8#@	6.9±0.8#@
Spleen nucleated cell number, 10^7/L	12	9.8±1.5	4.8±1.3*	4.3±1.5*	7.7±1.2#@	8.2±2.0#@
Bone marrow nucleated cell number, 10^6/L	12	45.1±3.2	14.0±1.0*	17.2±3.5*	33.3±5.2*#@	37.0±4.0#@
Bone marrow CFU	12	59.4±3.2	11.6±2.2*	22.1±3.6*	44.3±3.9*#@	49.3±3.9#@
Spleen CFU	12	93.2±4.1	40.0±5.4*	56.3±6.8*	88.3±6.8#@	87.6±4.7#@

Differences are statistically significant (p < 0.05) as compared

(*) — to the group of intact animals, (#) — to the control group (CP + normal saline)

(@) — to the group of animals treated with GSH

Effect of GSSG in combination with 0.1% cystamine on IL-2 and GM-CSF production by splenocytes, bone marrow, sple… blood cellular indices, and bone marrow and spleen hematopoietic colony formation capability in irradiated mice (M±m)

Parameter	n	Sham-irradiated animals	Irradiated animals			
		Normal saline	Normal saline	GSH	GSSG	GSSG+0.1% cystamine
…production by …ocytes, (U/mL)	12	45,4±4,2	5,6±0,71*	9,3±1,44*	29,3±3,18*#@	40,1±4,10#@
…F production by …plenocytes, …nies /10^5 cells)	12	132±11,8	28,6±4,5*	34,3±3,99*	103±11,6#@	113±9,07#@
Blo… …ocyte count, 10^9/L	12	13,3±1,08	3,1±0,9*	5,7±0,9*	9,3±4,5*#@	11,2±1,83#@
B… …mphocyte count, 10^9/L	12	8,6±0,74	3,38±0,61*	4,6±0,70*	6,79±0,82#@	7,12±0,899#@
…nucleated cell …mber, 10^7/L	12	10,5±0,97	5,8±0,9*	6,93±0,85*	8,9±1,07#@	10,7±1,13#@
Bo… …ow nucleated cell …mber, 10^6/L	12	48,3±3,8	15,1±1,69*	24,7±3,0*	39,5±4,17*#@	51,0±4,81#@
…marrow CFU	12	61,3±5,2	16,0±2,5*	25,6±3,99*	50,3±5,14*#@	55,7±5,31#@
…leen CFU	12	104±9,2	43,5±5,8*	66,3±7,07*	94,0±8,81#@	107±11,7#@

Diffe… are statistically significant ($p < 0.05$) as compared:

(*) … group of intact animals, (#) — to the control group (CP + normal saline);

(@) … group of animals treated with GSH.

Ta… Effect of GSSG in combination with 0.1% inosine on IL-2 and GM-CSF production by splenocytes, bone marrow, spleen ar… d cellular indices, and bone marrow and spleen hematopoietic colony formation capability in irradiated mice. (M±m)

…arameter	n	Sham-irradiated animals	Irradiated animals			
		Normal saline	Normal saline	GSH	GSSG	GSSG+0.1% inosine
… production by …cytes, (U/mL)	12	45,1±4,3	4,6±0,53*	9,9±1,08*	26,9±3,4$^{*\#@}$	44,3±4,71$^{\#@}$
…F production by …plenocytes, …ies /10^5 cells)	12	132±11,9	21,8±3,7*	35,9±4,15*	116±11,7$^{\#@}$	163±22,1$^{\#@}$
Blo… …kocyte count, 10^9/L	12	12,0+1,4	3,04±0,81*	4,95±0,62*	7,93±0,96$^{*\#@}$	10,9±2,04$^{\#@}$
Bl… …mphocyte count, 10^9/L	12	8,15+0,76	1,94±0,51*	4,0±0,58*	6,7±0,83$^{\#@}$	7,8±0,86$^{\#@}$
… nucleated cell …nber, 10^7/L	12	9,91+1,3	3,5±0,66*	5,5±0,70*	9,0±1,13$^{\#@}$	10,2±1,5$^{\#@}$
Bo… …row nucleated cell …nber, 10^6/L	12	47,3±3,18	13,0±1,8*	22,5±3,08*	39,9±4,5$^{*\#@}$	51,7±4,98$^{\#@}$
… marrow CFU	12	56,2+4,4	9,7±1,3*	25,3±3,7*	48,9±5,13$^{*\#@}$	69,0±7,03$^{\#@}$
…pleen CFU	12	154±9,45	35,0±5,14*	59,8±6,18*	99,3±10,11$^{\#@}$	167,0±17,3$^{\#@}$

Diffe… are statistically significant ($p < 0.05$) as compared:

(*) … group of intact animals; (#) — to the control group (CP + normal saline);

(@) … group of animals treated with GSH.

Tabl ffect of the test articles on number of normal lymphocytes per well (x10^4 cells) throughout the 96-hr incubation. (M±m)

Test articles (solutions)	24 hours	48 hours	72 hours	96 hours
GSSG in normal saline	27 ± 2	98 ± 6*	176 ± 12	386 ± 18*
GSSG + 0,003% H_2O_2	25 ± 4	108 ± 8*	231 ± 14*	419 ± 21*
GSSG + 0.1% inosine	28 ± 3	107 ± 5*	212 ± 16*	306 ± 12*
GSSG +0.1% cystamine	26 ± 3	93 ± 5*	186 + 10*	263 ± 14*
0,003 % H_2O_2	28 ± 2	73 ± 5	123 ± 8	206 ± 8
0.1% inosine	26 ± 4	78 ± 7	141 ± 12	216 ± 16
0.1% cystamine	30 ±2	72 ± 4	122 + 9	196 ± 11
10% fetal calf serum	29 ± 4	74 + 7	133 ± 18	263 ± 13

* Diff ...s are statistically significant ($p < 0.05$) as compared to 10% fetal calf serum.

2. Effect of the test articles on number of HL-60 cells per well (x10^4 cells) throughout the 96-hr incubation. (M±m)

Test articles (solutions)	24 hours	48 hours	72 hours	96 hours
GSSG in normal saline	102 ± 4	156 ± 6*	386 ± 21*	390 ± 11*
GSSG + 0,003% H_2O_2	96 ± 6*	132 ± 4*	286 ± 18*	306 ± 18*
GSSG + 0.1% inosine	49 ± 3*	76 ± 6*	138 ± 11*	165 ± 9*
GSSG +0.1% cystamine	68 ± 8*	102 ± 11*	242 ± 19*	256 ± 14*
0,003 % H_2O_2	122 ± 6	186 ± 12	488 ± 24	712 ± 22
0.1% inosine	96 ± 8*	152 ± 8*	312 ± 21*	527 ± 18*
0 1% cystamine	112 ± 10	182 ± 9	465 ± 11	618 ± 19
10% fetal calf serum	119 ± 7	181 ± 13	471 ± 7	752 ± 16

* D... ...s are statistically significant ($p < 0.05$) as compared to 10% fetal calf serum.

Tab… …fect of the test articles on the cytokine serum levels, the accumulation of ascitic fluid and the mean survival time of mice inoculated with leukemia L1210 cells (M±m)

Group … …nals	The number of injections	Concentration of factors in serum, (pg/mL);					Accumulation of ascitic fluid (weight gain, %)	Mean survival time
		IL-1	IL-2	IL-6	IFNα	TNFα		
	2	3	4	5	6	7	8	9
Cont… …ls	0	22,0±3,15	14.50±2.56	93.20±10.58	82.2±9.05	79.70±8.15	0,7±0,1	9.02±0.19
	3	28.5±4.01	23.18±3.11	108.0±14.12	100.55±11.34	80.3±8.81	7.14±0.9	
	7	13.4±2,68	17.8±2.51	136.70*±15.2	140.3±16.25	196.90±21.30	25.4±2.62	
Int… …s	0	20.09±1.95	13.14±1.12	84.0±9.65	108.0±11.33	77.90±6.85	0,2±0,1	35±0
	3	25.10±2.31	21.75±1.44	85.60±9.01	101.0±8.72	89.0±7.13	1.12±0.3	
	7	21.30±2.98	21.15±1.86	84.9±7.16	90.0±10.11	116.±10.83	4.6±1.23	
	0	27.5±3.60	14.7±3.13	124.40±13.7	144.80±15.34	98.10±11.54	0.77±0.16	10.74±0.51*
	3	57.6±7.14	57.7±6.80	301.0±32.2	508.0* ±54.3	397.0*±44.50	4.02*±0.53	
	7	167.5±18.30	144.5±17.03	678.±74.5	1207.0*±116.3	610.0*±71.9	15.67*±1.70	
GSSG … H_2O_2	0	19.8±2.05	14.84±2.13	108.0±9.17	119.40±9.56	78.0±6.15	0.44±0.16	11.13±0.49*
	3	126.0±13.9	99.0±11.3	298.±24.5	238.0±18.9	406.*±35.3	3.17*±0.41	
	7	123.5±12.7	189.0±21.4	445.±4.14	1413*±129	818*±73.5	14.04*±1.1	

Differenc… …istically significant ($p < 0.05$) as compared as compared to the control group

Table 13. (Continuation).

	2	3	4	5	6	7	8	9
	0	25.5±2.86	17.40±1.92	104.±8.15	122.4±10.43	121.9±10.33	0.63±0.16	
GSSG · osine	3	83.10±9.15	40.8±5.0	512.*±48.7	628.*±56.4	565.*±50.03	1.75*±0.25	12.01±0 49*
	7	238.0±29.56	91.1±11.08	106. ±9.14	1650.*±148	1904.*±186 0	5.69*±0.74	
	0	23.14±2.86	17.0±1.55	102.±8.04	129.0±9.80	101.5±8.16	0.76±0.19	
GSSG + amine	3	118.0±13.42	59.16±7.55	145.±11.8	761*±59.4	357.0*±28.30	2.47*±0.28	11 96±0 59*
	7	189.20±21.0	249.±22.7	400.0*±32.5	1700.*±163.	709.0*±59.0	6.85*±0.91	
	0	17.07±1.65	16.18±1.68	120.9±10.7	133.7±10.45	110.±9.13	0.79±0.17	
0 00	3	38.15±4 11	23.5±3.3	140.±13.3	189.±15.45	158.0±11.97	6.12±0.73	9 7+0 21
	7	23.6±3.05	45.5±5.8	103.±9.18	209.±18.30	220.0* ±24.5	21.61±2.55	
	0	41.0±4.23	17.80±1.49	108.±9.03	117.3±10.81	104.3±9.17	0.61±0.14	
0	3	55.6±6.17	22.3±2.14	91.0±8.8	160.0 ±12.47	130.0±10.85	7.02±0.64	9 61±0.18
	7	36.40±4.81	14.6±1.53	119.±10.5	205. ±21.3	157.0±15.80	26.30±2.57	
	0	36.0±3.12	16.9±1.5	63.0±5.0	115.0±10.52	88.6±5.19	0 47±0.18	
0 1 ... ie	3	47.50±5.17	17.30±1.46	70.0±12.6	200. ±18.0	185 0 ±16 70	5.93 ±0.47	9 53±0.18
	7	28.0±3.0	22.8±1.90	155.0±13.4	137.0 ±14.5	213.0 ±18 54	21.17±2.05	

Difference ... istically significant ($p < 0$ 05) as compared as compared to the control group

Tab e 'fect of the test articles on the cytokine serum levels, the accumulation of ascitic fluid and the mean survival time of mice inoculated with leukemia P388 cells (M±m)

Group als	The number of injections	Concentration of factors in serum, (pg/mL);					Accumulation of ascitic fluid (weight gain, %)	Mean survival time
		IL-1	IL-2	IL-6	IFNα	TNFα		
	2	3	4	5	6	7	8	9
Cont is	0	19.6±3.85	10.5±1.59	86.18±7.13	90.5±7.76	85.0±6.15	0.5±0.07	9,6±0.22
	3	34.7±5.42	26.7±3.18	133.0±15.2	113.0±12.0	96.17±8.2	6.9*±0.52	
	7	10.8±2.34	20.3±3.08	156.10*±20,0	158±10.8	218*±22.03	28.2*±2.9	
Inta s	0	25.12±1.76	17.70±1.84	104.50±9.94	90.50±7.19	88.64±7.14	0,3±0,2	35*±0
	3	33.0±3.57	26.8±3.07	92.80±8.03	116.0±10.55	89.0±7.23	1.62±0.4	
	7	30.83±2.15	25.40±2.17	102.0±8.89	112.31±10.8 6	93.7±7.64	5.1±1.08	
	0	23.5±4.22	12.8±1.95	102.0±12.8	134.±9.8	90.03±8.07	0.48±0.032	11.0±0.44*
	3	62.3±9.15	64.6±7.13	280.0*±31.2	460.* ±40.8	306.±24.4	3.7*±0.32	
	7	147.0±17.30	128.10±16.5 5	624.0*±45.6	1024. *±97.0	560.±48.8	15.2*±0.16	
GSSG H_2O_2	0	17.4±2.4	9.41±2.02	90.8±10.10	101.0±9.88	73.5±5.17	0.39±0.11	11.6±0.53*
	3	109.6±14.4	104.8±15.30	314.0±37.2	255.0*±22.3	355.*±36.2	2.93*±0.33	
	7	142.6±16.3	174.0±20.9	501.0*±48.3	1505*±131.0	890.*±78.3	13.6*±0.64	

Differences atistically significant ($p < 0.05$) as compared as compared to the control group

Table 14. (Continuation).

	2	3	4	5	6	7	8	9
GSSG · osine	0	28.7±3.05	7.13±0.98	129.8±14.0	123.4±10.01	109.0±11.2	0.56±0.16	12.7±0.51*
	3	75.0±8.13	36.4±4.8	618.0*±52.3	693.0*±61.8	517.* ±44.5	1.64*±0.19	
	7	210.4*±26.8	84.0±10.03	520.0*±51.0	1810.* ±129.	2120.* ±193.	5.15*±0.59	
GSSG + tamine	0	20.8±2.91	16.7±1.88	118.9±12.3	114.6±9.87	95.6±9.1	0.61±0.15	12.5±0.56*
	3	109.2±10.45	37.03±4.15	156.6±11.8	708.0*±61.9	326*±28.7	2.26*±0.17	
	7	168.0±21.15	211.0*±25.6	414.0*±18.4	1950*±180.0	785.*±69.0	6.08*±0.77	
0.0	0	15.5±2.04	14.95±2.16	134.0±15.6	129.±10.0	119.±9.13	0.63±0.15	9.9±0.24
	3	44.7±6.14	22.0±2.81	156.0±16.3	205.8±18.3	144.5±12.8	5.4±0.62	
	7	28.6±4.11	40.8±5.12	110.9±12.5	190.±16.7	248. ±20.7	20.3±2.28	
0.1	0	36.7±5.12	16.50±1.09	115.0±12.5	81.4±6.13	122.0±10.0	0.58±0.13	9.8±0.21
	3	48.2±7.13	20.19±1.54	90.0±7.11	105. ±11.3	96.5±8.7	6.8±0.8	
	7	31.0±5.12	13.40±1.68	129.0±10.4	184. ±16.1	144.8±12.9	25.0±2.22	
0.1% ne	0	30.0±4.02	14.9±2.05	72.7±9.10	107±8.06	80.5±7.14	0.67±0.22	9.93±0.27
	3	41.5±5.81	15.25±1.80	184.0±15.6	216. ±19.08	204. ±16.1	6.0 ±0.49	
	7	22.3±3.0	20.18±2.50	170.6±14.3	315. ±9.80	220. ±19.1	19.9±1.67	

Difference ... tistically significant ($p < 0.05$) as compared as compared to the control group

Table 15. Effect of GSSG administered intravenously on cytokine and erythropoietin serum levels in cancer patients

Patients	Number of injections	Serum level, pg/mL				
		IL-1β	IL-6	TNFα	INFα	erythropoietin
Pulmonary adenocarcinoma with pleural metastases	0	18.3	138.0	57.2	83.3	143.0
	3	96.7	156.0	280.0	395.6	605.0
	7	104.6	150.0	315.0	378.0	548.0
Stomach adenocarcinoma with liver metastases	0	12.0	93.5	27.0	4.6	21.6
	3	28.1	228.0	215.0	33.6	53.5
	7	31.7	204.0	147.0	34.0	47.1
Suprarenal corticocytoma with liver, pulmonary and peritoneal metastases	0	8.4	61.9	39.8	41.3	8.3
	3	12.9	105.0	113.0	56.0	32.4
	7	17.3	167.0	103.9	61.5	28.6

Table 16. Effect of GSSG on blood indices, cytokine and erythropoietin serum levels, and immunological parameters in patient with colorectal cancer and chemotherapy induced hemodepression

Parameter	Prior to the treatment	After the treatment completion
Erythrocytes	2.9×10^{12}/L	4.1×10^{12}/L
Hemoglobin	79 g/L	108 g/L
Leukocytes	3.6×10^{9}/L	5.4×10^{9}/L
Lymphocytes	0.67×10^{9}/L	1.57×10^{9}/L
Platelets	92×10^{9}/L	208×10^{9}/L
ESR	44 mm/hr	19 mm/hr
CD4⁺	204×10^{6}/L	609×10^{6}/L
CD8⁺	255×10^{6}/L	661×10^{6}/L
NK-cells	39×10^{6}/L	109×10^{6}/L
IL-1β	203 pg/mL	815 pg/mL
IL-6	318 pg/mL	1014 pg/mL
[illegible]	[illegible]	[illegible]
IFN[illegible]	84 pg/mL	[illegible]06 pg/mL
Erythropoietin	162 pg/mL	618 pg/mL

Table 17. Effect of GSSG on blood indices, cytokine and erythropoietin serum levels, and immunological parameters in patient with AIDS and cryptococcal meningitis

Parameter	Pre-treatment	Post-treatment
Erythrocytes	3.1×10^{12}/L;	3.9×10^{12}/L;
Hemoglobin	84 g/L;	126 g/L;
Leukocytes	6.3×10^{9}/L;	5.1×10^{9}/L;
Lymphocytes	0.8×10^{9}/L;	1.45×10^{9}/L;
$CD4^{+}$	55×10^{6}/L;	338.3×10^{6}/L;
$CD8^{+}$	135×10^{6}/L;	883×10^{6}/L;
IL-1β	18.9 pg/mL;	123.4 pg/mL;
IL-2	0.32 IU/mL	3.7 IU/mL
IL-6	16.0 pg/mL;	272.0 pg/mL;
IL-10	45.0 pg/mL;	608.0 pg/mL;
IFNα	27.0 pg/mL.	314.0 pg/mL.
IFNγ	15.7 pg/mL	349.8 pg/mL

Table 18. Effect of GSSG on blood indices, cytokine and erythropoietin serum levels, and immunological parameters in patient with AIDS and isosporiasis

Parameter	Pre-treatment	Post-treatment
Erythrocytes	4.04×10^{12}/L	4.75×10^{12}/L
Hemoglobin	108 g/L	129 g/L
Leukocytes	5.4×10^{9}/L	6.0×10^{9}/L
Lymphocytes	0.9×10^{9}/L	1.8×10^{9}/L
$CD4^{+}$	125×10^{6}/L	436.5×10^{6}/L
$CD8^{+}$	270×10^{6}/L	949.3×10^{6}/L
Total protein	46 g/L	78 g/L
IL-1β	27.8 pg/mL	202.4 pg/mL
IL-2	0.51 IU/ml	12.9 IU/ml
[illegible]	[illegible] pg/m[illegible]	[illegible]
[illegible]	[illegible] pg/m[illegible]	[illegible] pg/m[illegible]
IFNα	148.3 pg/mL	860.0 pg/mL
IFNγ	61.2 pg/mL	698.8 pg/mL

Table 19. Effect of GSSG on blood indices, erythropoietin serum level in patient with hypoplastic anemia and pancytopenia

Parameter	Pre-treatment	Post-treatment
Erythrocytes	1.8×10^{12}/L	4.3×10^{12}/L
Hemoglobin	43 g/L	119 g/L
Color index	0.72	0.83
Reticulocytes	0.22 %	2.85 %
Leukocytes	4.2×10^{9}/L	7.2×10^{9}/L
Lymphocytes	1.6×10^{9}/L	3.1×10^{9}/L
Platelets	72×10^{9}/L	219×10^{9}/L
ESR	46 mm/hr	15 mm/hr
Erythropoietin	9.2 pg/mL	201.7 pg/mL

Table 20. Effect of GSSH in combination with 0.1% inosine on blood and immunology indices and cytokine levels in patient with stomach cancer, peritoneal metastases, ascites and splenomegly.

Parameter	Prior to the treatment	2 months after the treatment beginning	4 months after the treatment beginning
Erythrocytes, 10^{12}/L	3,2	3,7	4,4
Hemoglobin, g/L	112	121	135
Platelets, 10^{9}/L	205	195	275
Leukocytes, 10^{9}/L	12.4	8,9	8,1
Neutrophils (stab),%	12	8	2
Neutrophils (segm.), %	54	44	47
Lymphocytes, %	21	36	41
Monocytes, %	8	7	9
Eosinophils, %	[illegible]	[illegible]	[illegible]
ESR, mm/hr	[illegible]	[illegible]	[illegible]
Total protein, g/L	62	76	82
Albumin, %	26	45	47

Table 20. (Continuation).

α1-globulin, %	3,0	7	11
α2- globulin, %	14,0	12	7
β- globulin, %	7	10	13
γ- globulin, %	50	26	22
A/G ratio	0.35	0.82	0,9
Urea, mmol/L	6.6	6.1	7.4
Creatinin, mmol/L	0,11	0,09	0,82
Bilirubin, mcmol/L	40,0	32,4	20,1
Bilirubin conjugated, mcmol/L	31,0	21,4	
Prothrombin index, %	75	79	95
Glucose, mmol/L	5,9	5,3	4,2
SGOT, mmol/hr/L	4,4	1,21	0,21
SGPT, mmol/hr/L	3,8	1,21	0,17
Lymphocytes, 10^6/L	260,4	3204	3321
B-lymphocytes ($CD20^+$)10^6/L	26	192	368
$CD4^+$-lymphocytes, 10^6/L	132.8	574	1024
$CD8^+$-lymphocytes, 10^6/L	13	374	908
$CD4^+$/$CD8^+$	10.2	1.5	1.1
IL2-receptor bearing cells ($CD25^+$), 10^6/L	26.8	498	2009
HLA11-receptor bearing cells, 10^6/L	13	258	754
NK-cells (CD16+), 10^6/L	26	324	576
IgA, g/L	3.2	2.38	2.38
IgM, g/L	3.6	0.58	1.42
IgG, g/L	21.82	14.34	12.2
Immune Complexes, OD units	337	216	117
IL-1β, pg./mL	92	727	813
IL-2 IU/mL	4.05	41.0	47.3
[illegible]	[illegible]	[illegible]	[illegible]
IFNα, pg./mL	70.8	672	604
IFNγ,pg./mL	105	624	519
TNFα, pg./mL	183	707	980

Table 21. Effect of GSSG in combination with 0.1% cystamine on blood and immunology indices and cytokine levels in patient with skin cancer (Merkel's cell carcinoma), local lymph node metastases and chemotherapy-induced hemo- and immunodepression.

Parameter	Prior to the treatment	3 months after the treatment beginning
Erythrocytes, 10^{12}/L	3,9	4,1
Hemoglobin, g/L	112	114
Platelets, 10^9/L	210	262
Leukocytes, 10^9/L	2.4	7.2
Neutrophils (stab),%	6	8
Neutrophils (segm.), %	79	60
Lymphocytes, %	8	24
Monocytes, %	4	7
Eosinophils, %	3	1
ESR, mm/hr	43	13
Total protein, g/L	61	78
α1-globulin, %	9.20	2.3
α2- globulin, %	12.32	8.2
β- globulin, %	13.08	14.0
γ- globulin, %	21.69	18.8
A/G ratio	0.78	0.94
Urea, mmol/L	8.54	4.3
Creatinin, mmol/L	0.123	0.095
Bilirubin, mcmol/L	4.6	4.1
Prothrombin index, %	82	100
Glucose, mmol/L	5.5	4.3
SGOT, mmol/hr/L	0.48	0.32
SGPT, mmol/hr/L	0.43	0.21
Lymphocytes, 10^6/L	192	1728
B-lymphocytes ($CD20^+$) 10^6/L	60	234
$CD4^+$-lymphocytes, 10^6/L	[illegible]	[illegible]
$CD8^+$-lymphocytes, 10^6/L	13	329
$CD4^+$/$CD8^+$	6.5	1.8
IL2-receptor bearing cells ($CD25^+$), 10^6/L	64	881

Table 21. (Continuation).

HLA11-receptor bearing cells, 10^6/L	36	498
NK-cells (CD16+), 10^6/L	24	624
IgA, g/L	4.9	5.2
IgM, g/L	0.99	1.24
IgG, g/L	24.3	15.6
Immune Complexes, OD units	264	111
IL-1β, pg./mL	156	637
IL-2, IU/mL	1.12	36.5
IL-6, pg./mL	244	1029
IFNα, pg./mL	79	513
IFNγ,pg./mL	58	234
TNFα, pg./mL	202	855

INTERNATIONAL SEARCH REPORT

International Application No
PCT/RU 96/00226

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 21368 A (LIFE SCIENCES TECHNOLOGIES) 10 December 1992 cited in the application see the whole document	1-3,9
A	WO 94 00141 A (BOEHRINGER MANNHEIM ITALIA) 6 January 1994 cited in the application see the whole document	1-9
A	EP 0 616 803 A (HOLT J.) 28 September 1994 see the whole document	1-9

[ ] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Date of the actual completion of the international search: 7 January 1997

Date of mailing of the international search report: 27.01.97

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer
Moreau,

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1 and 9
   because they relate to subject matter not required to be searched by this Authority, namely:
   Remark: Although claims 1 and 9 are directed to a method of treatment of of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/RU 96/00226

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